Enterobacteriaceae in processed cocoa products

Enterobactérias em produtos processados de cacau

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Maristela da Silva do NASCIMENTO[1]*, Érika Marques REOLON[1], Aline Regina Barbosa SANTOS[1], Vanessa Eliana MOREIRA[1], Ivone Francisca da SILVA[2], Neusely da SILVA[1]

*Endereço para correspondência: [1]Instituto de Tecnologia de Alimentos, Campinas, SP, Brasil, tel: 19 3743 1815, fax: 19 3743 1812, e-mail: mnascimento@ital.sp.gov.br.

[2]Departamento de Ciências de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Campinas/SP, Brasil.

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ABSTRACT

Little is known of the presence of *Salmonella* in Brazilian cocoa, which justifies the present work that had the aim of checking the presence of total *Enterobacteriaceae*, coliforms, *Escherichia coli* and *Salmonella* in semi-processed cocoa products. A total of 150 samples of cocoa products from two different cocoa-processing manufacturers were analyzed (30 samples of nibs, 30 of liquor, 30 of cocoa cake, 30 of cocoa butter and 30 of cocoa powder). The samples of processed cocoa products had pH values between 5.31 and 7.35, and water activity ranging from 0.29 to 0.52. *E. coli* and *Salmonella* were not detected in any of the samples analyzed. Regarding the other analyzed microorganisms, 17% of the nibs contained total Enterobacteriaceae, 20 % showed total coliforms, while thermotolerant coliforms were detected in one sample (0.6 log MPN/g). Seven percent of liquor and cocoa powder samples showed total coliforms. In cocoa cake, the same percentage in regard to total *Enterobacteriaceae* was observed. Total *Enterobacteriaceae* and total coliforms were detected in one sample of cocoa butter. Despite the low contamination observed, these results indicate failure in quality programs of the manufactures studied, since these bacteria are easily inactivated by thermal and sanitizer process.

Keywords. cocoa, *Salmonella*, coliform, *Escherichia coli*, chocolate.

RESUMO

Pouco se sabe sobre a presença de *Salmonella* no cacau brasileiro, o que justifica o presente trabalho cujo objetivo foi verificar a presença de enterobactérias totais, coliformes, *Escherichia coli* e *Salmonella* em derivados processados do cacau. Foram analisadas 150 amostras de produtos derivados do cacau provenientes de duas indústrias processadoras (30 amostras de "nibs", 30 de liquor, 30 de torta, 30 de manteiga de cacau e 30 de cacau em pó). As amostras apresentaram valores de pH entre 5,31 e 7,35 e atividade de água entre 0,29 e 0,52. Não foi detectada *E. coli* e *Salmonella* em nenhuma amostra analisada. Com relação aos demais grupos de microrganismos analisados, 17% das amostras de nibs apresentaram enterobactérias totais, 20% apresentaram coliformes totais e em uma amostra foram detectados coliformes termotolerantes (0,6 log NMP/g). Sete por cento das amostras de liquor e cacau em pó apresentaram coliformes totais. Nas amostras de torta foi observada a mesma porcentagem para enterobactérias totais. Em uma amostra de manteiga de cacau foram detectados enterobactérias totais e coliformes totais. Apesar da baixa contaminação observada, esses resultados indicam falhas no programa de qualidade das indústrias avaliadas, já que essas bactérias são facilmente inativadas por processo térmico e sanitizante.

Palavras-chave. *Salmonella*, coliforme, *Escherichia coli*, cacau, chocolate.

INTRODUCTION

The first step in conventional cocoa processing consists of roasting the cleaned, whole beans in their shells at a temperature between 120 and 150 ºC, for 5 to 120 minutes. During roasting, a series of chemical reactions critical to the development of aroma, taste and color of the chocolate take place. The time/temperature combination will depend on several factors, including the origin and harvesting time within the year, pre-roasting treatments, the moisture level, the size of the beans and the desired flavor characteristics. Upon completion of roasting, the beans are cooled and cracked. Next, the shell and germ are removed ("winnowing") to obtain the nibs[1].

The nibs are ground to a fine particle size, called cocoa mass or cocoa liquor. Cocoa mass is subjected to hydraulic pressing, which separates some of the cocoa butter from the solid cocoa mass, resulting in compressed cocoa cake. Cocoa butter is the most valuable by-product of the pressing process. Cocoa cake is normally submitted to one more grinding process to obtain a fine powder of different particle sizes. Cocoa powder can be used in the manufacture of chocolate-flavored beverages, confectionery and bakery fillings and ice cream[1].

From a public health standpoint, the microorganisms most likely to adversely affect the quality of chocolate are enteropathogens like *Salmonella*. With regard to *Salmonella*, the European Union[2] lists chocolate among the products associated with major salmonellosis outbreaks in humans that spread across several countries and affected large numbers of people. Although cocoa products are not the only ingredients that may introduce *Salmonella* into chocolate, they have been implicated as the most prominent potential source of some outbreaks (dried cocoa beans, cocoa powder). Cordier[3] points out cocoa beans as a major source of *Salmonella* contamination throughout the manufacturing of chocolate and cocoa-based ingredients. Consequently, it is imperative that in-process products be routinely screened for the presence of *Salmonella* as part of an integrated control process that begins with the selection of reliable suppliers[4].

Indicator microorganisms are generally used to measure the quality of the practices used to ensure proper processing. Total *Enterobacteriaceae* are used as an indicator of hygiene practices since they are easily inactivated by sanitizers, and are able to colonize several niches of food processing plants[5].

The coliform group is a subgroup of *Enterobacteriaceae*, and the most used as an indicator in the food industry. They are aerobic or facultative anaerobic Gram-negative, nonspore-forming rods that ferment lactose, forming acid and gas. Representatives of 20 or more species may conform to criteria for this group, including fecal (*Escherichia coli*) and non fecal origins (*Citrobacter*, *Enterobacter*, etc)[5].

Nonetheless, there is still a huge gap in the scientific data and information available concerning contamination of primary ingredients. Thus, the implementation of any prevention measures should be preceded by detailed studies that allow determining prevalence and the main points of entrance of these microorganisms into the chocolate production chain. For this reason, the aim of this study was to investigate the presence of total *Enterobacteriaceae*, coliforms, *Escherichia coli* and *Salmonella* in semi-processed cocoa products.

MATERIAL AND METHODS

Sampling

A total of 150 samples divided into five groups of semi-finished processed cocoa products from two different processing Brazilian manufacturers were analyzed. Each group consisted of 30 samples of each product type: nibs, cocoa liquor, cocoa cake, cocoa butter and cocoa powder.

Test methods

Water activity was measured at 25 ºC with a hygrometer Aqua Lab 3TE (Braseq, Brazil). The pH was determined according to the method developed by the Instituto Adolfo Lutz[6]. *Salmonella* analysis was performed according to the method of the Bacteriological Analytical Manual (BAM/FDA)[7]. The pre-enrichment phase was performed in reconstituted skimmed milk to 10%, supplemented with brilliant green. After that, the sample materials were enriched in Rappaport–Vassiliadis and tetrathionate broth and subsequently plated onto Bismuth Sulphite Agar, Hecktoen enteric agar, and Xylose Lysine Deoxycholate agar. Suspect colonies were confirmed by biochemical and serological tests. *Enterobacteriaceae* were enumerated on Violet Red Bile Glucose agar by the plate count method. Total coliforms, thermotolerant coliforms and *E. coli* counts were determined by the Most Probable Number (MPN) technique, described in the Compendium of methods for microbiological examination of foods[8]. The presumptive test was performed using Lauryl Sulfate

Tryptose broth. Confirmation of total coliforms was carried out in Brilliant Green Bile broth and that of thermotolerant coliforms in EC broth. Confirmation of *E. coli* was followed by isolation on EMB agar and confirmation by biochemical tests (Indole, Voges–Proskauer, Methyl Red and citrate).

RESULTS

Tables 1 and 2 show the results for water activity, pH and microbiological analyses of 150 samples of processed cocoa products from two cocoa processing manufacturers. *Salmonella* and *E. coli* were not detected in any of the 150 samples (date not shown).

Table 1. Results of pH and water activity of processed cocoa products

Product	pH	Water activity
Nibs	4.98 to 5.67	0.42 to 0.57
Liquor	5.54 to 7.12	0.22 to 0.80
Cocoa cake	5.40 to 8.48	0.24 to 0.48
Cocoa butter	5.51 to 7.87	0.41 to 0.56
Cocoa powder	5.57 to 8.24	0.28 to 0.58

Table 2. Results of microbiological analyses of processed cocoa products

Product	Contamination parameter	Total *Enterobacteriaceae* (Log CFU/g)*	Total coliforms (Log MPN/g)**	Thermotolerant coliforms (Log MPN/g)**
Cocoa nibs	Positive samples (%)	05 (17%)	06 (20%)	01 (3%)
	Counts in positive samples (mean)	1.0 to 2.0 (1.2)	0.6 to 1.4 (0.9)	0.6
Cocoa liquor	Positive samples (%)	0	02 (7%)	0
	Counts in positive samples (mean)	-	0.6 to 1.0 (0.8)	-
Cocoa butter	Positive samples (%)	01 (3%)	01 (3%)	0
	Counts in positive samples (mean)	1,0	0.6	-
Cocoa cake	Positive samples (%)	02 (7%)	0	0
	Counts in positive samples (mean)	1.0 to 1.3 (1.2)	-	-
Cocoa powder	Positive samples (%)	0	02 (7%)	0
	Counts in positive samples (mean)	-	0.6 to 0.9 (0.7)	-

*Colony forming units, detection limit 1.0 Log CFU/g. **Most probable number, detection limit 0.5 Log MPN/g

The cocoa nib samples had water activity values between 0.422 and 0.567 and the pH varied between 4.98 and 5.67. Five (17%) of the 30 samples were found to be contaminated with total *Enterobacteriaceae*, with counts varying from 1.0 to 2.0 Log CFU/g. Total coliforms were detected in six (20%) of the 30 samples, with mean of 0.9 Log MPN/g, while thermotolerant coliforms were detected in one sample (0.6 Log MPN/g).

The samples of cocoa liquor had water activity values between 0.22 and 0.80 and the pH varied between 5.54 and 7.12. Total *Enterobacteriaceae* were not detected

by the plate count method. However, total coliforms were isolated in two (7%) of the 30 samples by the MPN technique, with counts of 0.6 and 1.0 Log MPN/g.

The samples of cocoa butter had water activity values ranging between 0.41 and 0.56 and the pH varied between 5.40 and 8.48. One sample (3%) was found to be contaminated with total *Enterobacteriaceae* (1.0 Log CFU/g). In another sample (3%) total coliforms were detected, with a count of 0.6 Log MPN/g.

The cocoa cake samples had water activity ranging between 0.24 and 0.35. The pH varied between 6.60 and 7.35. Total *Enterobacteriaceae* were observed in two samples (7%), with counts of 1.0 and 1.3 Log CFU/g. However, total coliforms were not isolated in any analyzed sample.

The samples of cocoa powder had water activity between 0.28 and 0.58. The pH varied between 5.57 and 8.24. Just as occurred for liquor samples, total *Enterobacteriaceae* were not detected in cocoa powder by the plate count method. Nevertheless, in two samples (7%), using the MNP technique, contamination by total coliforms was observed, with counts varying from 0.6 to 0.9 Log MPN/g.

The fact of total *Enterobacteriaceae* not being detected in samples which showed contamination by total coliforms was possibly due to the difference between methods and detection limit used in this study. Total *Enterobacteriaceae* were analyzed by the plate count method, whose detection limit is 1.0 Log CFU/g. In contrast, the MPN method was used to isolate total coliforms which a part from having a lower detection limit (0.5 Log MPN/g), also has a step in nonselective broth that allows for the recovery of possibly injured cells. Furthermore, it is worth pointing out that the total coliform count observed in liquor and cocoa powder samples were below the detection limit of the total *Enterobacteriaceae* method (1.0 Log CFU/g).

DISCUSSION

The processing of cocoa beans into semi-processed products consists basically of the following steps: bean selection and cleaning, roasting, grinding, pressing and pulverizing. Roasting is the main step capable of significantly reducing the microbial counts, even though alkalinization also serves as a barrier function.

The low water activity and the high fat level of some semi-processed cocoa products may influence the increase in heat resistance of the pathogens[4]. According to Jay[5], the optimum pH value for *Enterobacteriaceae* growth falls within the neutral range (6.6 to 8.2); values above 9 and lower than 4 are considered bactericidal. With regard to water activity, growth inhibition of most members of this family occurs at values lower than 0.94. All the products tested had a pH falling within the development range for *Enterobacteriaceae* such as *Salmonella*, but at the same time had water activity values lower than 0.60 which is an adverse growth condition. However, the fact that the semi-processed cocoa product exhibited low water activity values does not prevent the contamination with and the subsequent survival of these microorganisms, what was observed in this study.

In all 150 samples of semi-processed cocoa products analyzed, *Salmonella* and *E. coli* were not detected. However, 18.5% of the samples showed total *Enterobacteriaceae* and/or total coliforms, microorganisms routinely used as hygiene indicator of process[5]. Hence, despite the low contamination (≤2 Log CFU or MPN/g), this result suggests failure in quality programs, since these bacteria are easily inactivated by thermal and sanitizer process.

It is worrying from public health point of view because a possible contamination by *Salmonella* and *E. coli* could occur in these conditions. As these products are used as raw material for chocolate manufacturing, the control must be rigorous, seeking the absence of indicator organisms. This concern is corroborated by the fact that two salmonellosis outbreaks associated with chocolate have been reported, in which the source of contamination was traced back to the cocoa beans or the cocoa powder[9]. In addition, epidemiological surveys have confirmed that the *Salmonella* infecting dose in this type of product is very low, sometimes even lower than 1 CFU/g[9].

Therefore, temperature monitoring during cocoa roasting, use of quality raw material, use of good manufacturing practices, employee training, pest control, and adequate, well-executed hygiene program are prime factors throughout the supply chain of cocoa and chocolate to obtain products microbiologically safe.

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REFERENCES

1. Beckett ST. The science of chocolate, 2nd ed. London (UK): Royal Society of Chemistry Paperbak; 2008.
2. European Commission. *Salmonella* in Foodstuffs. Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health. [cited 2007 nov 22]. Avaliable from: http://ec.europa.eu/food/fs/sc/scv/out66_en.pdf.
3. Cordier JL. HACCP in the chocolate industry. Food Contr. 1994;5:171-5.
4. International Commission on Microbiological Specifications for Foods. Cocoa, chocolate and confectionery. *In*: Microorganisms in Foods 6: Microbiological Ecology of Food Commodities. 2nd ed. New York (NY): Kluwer Academic; 2005. p. 467-479.
5. Jay JE. Microbiologia de alimentos. 6th ed. Porto Alegre (RS): Artmed, 2005.
6. Instituto Adolfo Lutz (São Paulo – Brasil). Métodos Físico-Químicos para Análise de Alimentos: Normas Analíticas do Instituto Adolfo Lutz. 4th ed. Brasília (DF): ANVISA; 2005.
7. Andrews WH, Hammack TS. *Salmonella*. *In*: Bacteriological analytical manual online. Food and Drug Administration. [cited 2007 nov 22]. Avaliable from: http://www.cfsan.fda.gov/ebam/bam-5.html.
8. Kornacki JL, Johnson JL. *Enterobacteriaceae*, coliforms and Escherichia coli – quality and safety indicators. *In*: Downes, FP, Ito K, editors. Compendium of methods for the microbiological examination of foods. Washington (DC): American Public Health Association; 2001. p.69-82.
9. Werber D, Dreesman J, Feil F, van Treeck U, Fell G, Ethelberg S et al. International outbreak of *Salmonella* Oranienburg due to German chocolate. BMC Infect Dis. 2005;5(7):7-17.